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Phenotypic Directed Antibody Selection

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In this issue of *Chemistry & Biology*, Xie and colleagues describe a "phenotype directed" approach to identify antibodies that protect cells from death caused by rhinovirus infection. The cellular antibody library of 10^8 clones yielded two antibodies that prevented cell death via the same viral target: rhinovirus 3C protease.

Over the last two decades, recombinant antibody selection technologies such as phage and yeast display have provided powerful methods to generate novel antibodies. Using antibody display technologies, valuable functional antibodies have been identified that interfere in cell signaling by blocking or activating extracellular receptor signaling. Such antibodies are initially identified on the basis of binding a target antigen before being individually produced and tested in cell-based functional assays.

The ready availability of antibody genes using recombinant antibody approaches opens up the possibility of combining antibody expression/production with functional "reporting" within the same cell. A number of recent publications have described the identification of functional antibodies based on this novel approach. Antibody gene populations are introduced into reporter cells, and clones within the resulting population of cells with altered phenotypes can be identified. It is then relatively straightforward to recover the antibody gene causing the modified cellular behavior.

Typically, a molecular target is identified, and a target-specific antibody population is generated (e.g., from phage display) and introduced into the reporter cells (Figure 1, Target Directed). The reporter cells then allow identification or selection of clones within the population with an antibody-directed alteration in phenotype (e.g., altered gene expression or survival). To work efficiently, it is necessary to have a method to introduce one or a few antibody genes per cell (e.g., by lentiviral infection). It is also necessary for the expressed antibody to be attached or retained close to the cell producing it to retain a linkage between phenotype and genotype. This has been achieved previously either through tethering the antibody to the cell surface or through the use of semisolid medium to retain secreted antibodies in the vicinity of producing cells.

Through this approach, agonistic antibodies targeted to the erythropoietin receptor, the thrombopoetin receptor, and the GCSF receptor have been identified using reporter cells transfected with the target gene (Xie et al., 2013; Zhang et al., 2012, 2013). In a related approach, antibodies that neutralized FGF4 or blocked its receptor FGFR1 were identified through regulated, secreted expression of antibody populations in mouse embryonic stem cells (Melidoni et al., 2013). Changes in differentiation outcomes were identified using lineage-specific GFP expression.

In contrast to this "target-directed" approach, Xie et al. (2014); this issue of *Chemistry & Biology*) have used an approach that makes no assumptions about the target antigen (Figure 1, Phenotype Directed). A similar nontargeted approach has previously led to the identification of antibodies that activated integrin signaling through "RGD" sequence motifs within their CDR regions (Yea et al., 2013). The lack of a predefined target using the "phenotype-directed" approach increases the level of difficulty and places a greater requirement on achieving the largest possible library. Xie et al. (2014) used lentiviral infection to introduce an unbiased antibody library into HeLa cells, creating a cellular library of 10^8 clones. Phenotypic selection for rare events within a large library requires a dominant phenotype for screening or selection, and there is none better than selection for cell survival. In this case, antibodies were identified that imparted resistance to cell death caused by rhinovirus infection.

In the work of Xie et al. (2014), antibodies were expressed within the cytoplasm, which not only ensures a close linkage between "phenotype" and "genotype" within the same cell, but also opens up a "universe" of intracellular targets. Expression of antibodies within the cytoplasm, however, brings its own problems. Only a small proportion of antibodies are correctly folded in the reducing environment of the cytoplasm (Shaki-Loewenstein et al., 2005), so the "effective" library size will be significantly less than the 10^8 clones created. The efficiency of this approach could be enhanced using antibody frameworks or, indeed, other binding scaffolds that can fold effectively in the reducing environment of the cytoplasm. Despite these potential limitations, Xie et al. (2014) identified two different antibodies that protected HeLa cells from rhinovirus infection. This is impressive, given the need for a highly effective blockade of function within an antibody-expressing cell clone against a backdrop of rampant viral infection.

Having identified functional antibodies by this "phenotype-directed" approach, the target was identified as the rhinovirus 3C protein (using the antibodies for affinity capture coupled to mass spectrometry). 3C protease is known to play an important role in rhinoviral replication. The two selected antibodies bound different epitopes of the 3C protease and appear to operate by different mechanisms, with only one of them directly affecting enzymatic activity in biochemical assays.

The "phenotype-directed" approach uses a library of antibody genes to knockout gene function at the protein level. Development of genetic-based methods for gene knockout, such as the recently described CRISPR/Cas9 system, are progressing at a breathtaking pace (Shalem et al., 2014; Wang et al., 2014), and these can identify functionally important genes with relative

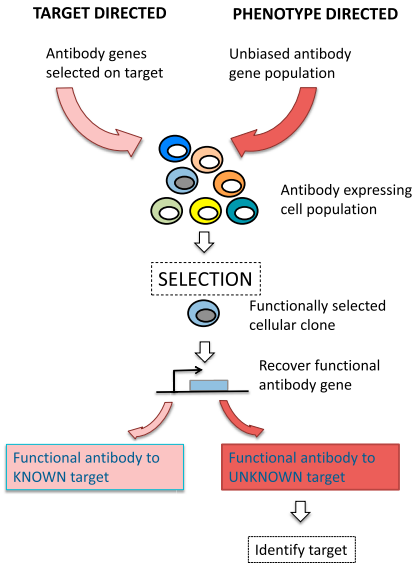

Figure 1. Alternative Approaches to Identify Functional Antibodies

"Target-directed" approach depends on knowing the identity of the target and introducing a preselected population of binders into mammalian reporter cells. "Phenotype-directed" approach, employed by Xie et al. (2014), introduces a "naive" antibody population and selects desired antibody clones based on the specific phenotype they elicit. Target identification in this scheme is the final step taken to resolve the antibody's mechanism of action.

ease. Identification of functionally important genes can also emerge from existing knowledge of biological systems. In the case of rhinovirus infection, it could be argued that 3C protease as a target was predictable based on prior knowledge of viral biology. Prior knowledge of the role of a gene, however, doesn't necessarily highlight it as the "Achilles heel" for targeting at the protein level. In addition, functional knockout at the protein level permits a more subtle intervention than the "all or nothing" effect of DNA based knock-outs. Thus, protein knock-outs can act as a useful complement to the increasingly efficient DNA-based approaches. Furthermore, the availability of functional antibodies provides the potential for functional knockdown in nongenetically modified systems through exogenous addition of antibody.

Although intracellular antibodies may help identify points of biological intervention, to date there are no examples of antibody therapeutics directed to intracellular targets, and extracellular receptors and ligands remain the dominant target class for therapeutic intervention. Whether intracellular or extracellular proteins are targeted, realizing the potential of phenotype-directed selection schemes requires construction of large mammalian cell libraries combined with powerful selection or reporting systems to identify functional blockers or activators. In this study, cell survival was used to identify antibodies of interest. The development of phenotype-directed approaches to identify binder/target combinations effecting changes in more subtle cellular responses remains a challenge. For example, identification of antibodies that *cause* cell death could identify targets for antibody-directed therapies in areas such as oncology. Thus, establishment of creative cellular screens based on parameters other than cell survival represents an important challenge and opportunity in target/drug discovery using phenotypic-directed selection.

REFERENCES

Melidoni, A.N., Dyson, M.R., Wormald, S., and McCafferty, J. (2013). Proc. Natl. Acad. Sci. USA *110*, 17802–17807.

Shaki-Loewenstein, S., Zfania, R., Hyland, S., Wels, W.S., and Benhar, I. (2005). J. Immunol. Methods *303*, 19–39.

Shalem, O., Sanjana, N.E., Hartenian, E., Shi, X., Scott, D.A., Mikkelsen, T.S., Heckl, D., Ebert, B.L., Root, D.E., Doench, J.G., and Zhang, F. (2014). Science *343*, 84–87.

Wang, T., Wei, J.J., Sabatini, D.M., and Lander, E.S. (2014). Science *343*, 80–84.

Xie, J., Zhang, H., Yea, K., and Lerner, R.A. (2013). Proc. Natl. Acad. Sci. USA *110*, 8099–8104.

Xie, J., Yea, K., Zhang, H., Moldt, B., He, L., Zhu, J., and Lemer, R.A. (2014). Chem Biol. *21*, this issue, 274–283.

Yea, K., Zhang, H., Xie, J., Jones, T.M., Yang, G., Song, B.D., and Lerner, R.A. (2013). Proc. Natl. Acad. Sci. USA *110*, 14966–14971.

Zhang, H., Wilson, I.A., and Lerner, R.A. (2012). Proc. Natl. Acad. Sci. USA *109*, 15728–15733.

Zhang, H., Yea, K., Xie, J., Ruiz, D., Wilson, I.A., and Lerner, R.A. (2013). Chem. Biol. *20*, 734–741.